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N and P combined addition accelerates the release of litter C, N, and most metal nutrients in a N-rich subtropical forest

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Abstract

Imbalanced nitrogen (N) and phosphorus (P) depositions are profoundly shifting terrestrial ecosystem biogeochemical processes. However, how P addition and its interaction with N addition influence the release of litter carbon (C), N, P, and especially metal nutrients in subtropical forests remains unclear. Herein, a two-year field litterbag experiment was conducted in a natural subtropical evergreen broadleaved forest of southwestern China using a factorial design with three levels of N addition (0, 10, and 20 g N m^{-2} y^{-1}) and P addition (0, 5, 15 g P m^{-2} y^{-1}). During two years of decomposition, N- and P-only addition treatments decreased the accumulated mass loss and release rates of litter C, N, P, K, Na, and Mn ($p < 0.05$); N and P coaddition treatments increased the accumulated mass loss and release rates of litter C, N, K, Na, Mn, and Cu ($p < 0.05$) and decreased the accumulated release rates of litter P and Mg ($p < 0.05$); the C/P and N/P ratios of the residual litter increased under the N-only addition treatments ($p < 0.05$) and decreased under the P-only addition and N and P coaddition treatments ($p < 0.05$). Overall, the results suggest that combined N and P supply can increase biological activities and thus accelerate the release of litter C, N, and most metal nutrients, as expected within the framework of ecological stoichiometry and growth rate hypothesis. Our study also highlights that the N addition effect on litter C and nutrients release depends on P availability.

Keywords Nitrogen and phosphorus imbalance; Stoichiometry; Litter nutrient release; Soil fauna; Phosphorus addition; Biogeochemistry.

Introduction

Forest litter on Earth stores approximately 2,830 Tg of carbon (C); however, 220 Tg of carbon dioxide is released into the atmosphere each year through the process of litter decomposition (Bani et al., 2018). In addition, litter nutrients (e.g., nitrogen (N), phosphorus (P), and metal elements) can return to soils via nutrient release processes, maintaining the stability of the soil nutrient pools (Austin and Vivanco, 2006; Bani et al., 2018). Therefore, elucidating the mechanisms associated with the release of C and nutrients from forest litter is crucial for understanding their dynamics in global biogeochemical cycle (Cook-Patton et al., 2020; Friedlingstein et al., 2020).

Nitrogen and P are two of most important limiting nutrients in forest ecosystems, which controls biogeochemical cycles in forests (Du et al., 2020; Sardans et al., 2011). Atmospheric N deposition has been increasing at an unprecedented rate in human history since the Industrial Revolution (Galloway et al., 2008). However, this enhancement has not occurred in parallel with P deposition, resulting in an imbalanced N and P deposition (Du et al., 2020; Fisher et al., 2012; Zhu et al., 2016a). In this context, an increasing number of studies have shown that changes in atmospheric N and P deposition and their imbalances significantly alter biodiversity (Peñuelas and Sardans, 2022; Tie et al., 2021), net primary productivity (Elser et al., 2007), and even the fate of nutrient contents (van Huysen et al., 2016; Zeller et al., 2001) in forest ecosystems.

The effect of N addition on the elemental release from litter is complex and unpredictable (Keeler et al., 2009; Trentini et al., 2018). Globally, the results from field experiments have shown inconsistencies; studies have either identified positive effects (Jing et al., 2019), negative effects (Bubier et al., 2007; Dias et al., 2013; Zhu et al., 2016a), or neutral effects (Zhuang et al., 2020) of N addition on the release of litter C and nutrients. The response of litter decomposition to N addition is mostly related to the level of fertilization and the availability of soil N. For example, a recent meta-analysis reported that low level of N addition accelerates the C release from litter, while high level of N addition inhibits litter decomposition due to decreasing soil pH (Su et al., 2022). Besides, N addition usually increases microbial diversity and C-hydrolase activity in forest ecosystems with low soil N availability, thereby accelerating the degradation of refractory components and the release of litter C (Allison et al., 2009; Jing et al., 2019). In contrast, in high soil N availability forest ecosystems, N addition can exacerbate nutrient imbalances and reduce microbial abundance, thus inhibiting litter C biodegradation (Bubier et al., 2007; Zhou et al., 2021). Moreover, the effect of N addition on the release of litter C and nutrients also varies with the climatic conditions (Su et al., 2022), litter quality (e.g., C/N ratio and lignin) (Song et al., 2019; Valera-Burgos et al., 2013), decomposition stage (Allison et al., 2009; Peng et al., 2022), and vegetation types (Peng et al., 2019; Yan et al., 2020).

In comparison with N addition effects, relatively few studies have examined the effects of P addition on the release of litter C and nutrients, and the existing studies have produced inconsistent results. For instance, Baiocchi Jacobson et al. (2011) and Chen et al. (2016b) reported that P addition has increased litter C decomposition rates in tropical forests. In contrast, van Huysen et al. (2016) found that P addition did not alter litter C and N release rates in a temperate forest. Furthermore, N and P usually interact (Chen et al., 2016a; Elser et al., 2007), which increases the uncertainty of the responses of litter nutrient release rates to N and P additions. For example, the combined addition of N and P can increase nutrient availability on the surface of decomposed litter, which interactively affects the rate of litter decomposition via regulating microbial activity and productivity (Hattenschwiler and Jorgensen, 2010; Tie et al., 2022; van Huysen et al., 2016). However, the results of N and P combined addition on litter C decomposition rate are inconsistent in different ecosystems, including positive (Chen et al., 2016b; Peguero et al., 2019) and neutral effects (Chen et al., 2013). Notwithstanding, prior studies have mainly focused on the effect of N and/or P addition on litter C loss in tropical and temperate forests (Bubier et al., 2007; Jing et al., 2019; Zhang et al., 2020; Zhu et al., 2016b). However, the mechanisms by which P addition and its interaction with N addition influence the release of litter N, P, and especially metal nutrients (e.g., manganese, which has been shown to be critical for litter decomposition (Berg, 2014)) in subtropical forests have rarely been studied.

Natural subtropical evergreen broadleaved forests are the predominant forests in the rainy zone of southwestern China (one of the main areas of China containing these forests) (Tie et al., 2022; Zhang, 2006). This rainy area has accumulated large amounts of N since atmospheric wet N deposition (9.0 g N m^{-2} y^{-1} at present) increased approximately 2-fold over the past four decades (since 1980) (Tu et al., 2014; Yu et al., 2019), and these deposition levels are expected to continue rising until at least 2050 because of the rapid industrial development and the increasing application of fertilizers in this area (Zhu et al., 2016a). The soils in natural evergreen broadleaved forests of this rainy area are typically N-rich and have a low pH (approximately from 4.0 to 4.4) (Peng et al., 2020; Tie et al., 2020). However, it is still unclear how increasing the available N and P for microbial productivity impacts the release of litter C and nutrients in these forests. Here, a two-year field litterbag experiment was conducted in a natural evergreen broadleaved forest in the rainy zone of southwestern China using a factorial design with three levels of N addition (0, 10, and 20 g N m^{-2} y^{-1}) and P addition (0, 5, 15 g P m^{-2} y^{-1}) to examine the effects of N and P addition on the release of litter C, N, P, and metal nutrients. Prior studies highlight that, in N-rich forests, N addition can exacerbate the imbalance of N and P and thus usually inhibits biodegradation (Bubier et al., 2007; Zhou et al., 2021). Moreover, according to the ecological stoichiometry and growth rate hypothesis (Elser et al., 2007; Sardans et al., 2011), maximum decomposition rates can be observed when N and P are both at high availability and meet the needs of the decomposers (Craine et al., 2007). We, therefore, considered the following hypotheses: **i)** the addition of only N will inhibit litter C decomposition and will decrease litter nutrient release rates in our study forest, while **ii)** the addition of P in combination with N will synergistically increase the release rate of litter C and nutrients.

Materials and methods

Study site description

This study was conducted at the field monitoring station of a natural evergreen broad-leaved forest in the center of the rainy zone of southwestern China (Fig. S1A and B; 103°0'25"E, 30°4'6"N; 970 m a.s.l.). The study site has a subtropical humid monsoon climate with a mean annual temperature of 16.1 °C and a mean annual precipitation of 1700 mm (Wei et al., 2020). In the studied forest, *Schima superba*, *Quercus serrata*, and *Symplocos botryantha* are the dominant tree species, with the average tree composition being 60% *S. superba*, 20% *Q. serrata*, and 10% *S. botryantha*, respectively. The soil is an old alluvial yellow loam classified as a Ferralsol (WRB, 2015). The average thickness of the surface organic layer is approximately 3 cm, and the soil depth to bedrock is >80 cm. The topsoil (0-20 cm) pH, organic C, total N, and total P were 4.18 ± 0.05, 30.8 ± 1.88 mg g^{-1}, 2.15 ± 0.22 mg g^{-1}, and 0.191 ± 0.019 mg g^{-1}, respectively (mean ± standard deviation). The annual average atmospheric N and P wet deposition in the studied forest was approximately 9.0 g N m^{-2} y^{-1} and 0.10 g P m^{-2} y^{-1}, respectively (Tu et al., 2014; Zhu et al., 2016a). A more detailed description can be found in our prior studies (Tie et al., 2022; Tie et al., 2021).

Experimental design

Twenty-seven 5 × 5 m plots were established in the study forest, and the plots were placed randomly with three plots for each treatment (N and/or P addition treatments, see below). Each plot was separated by a distance >5 m from other plots. Twenty-four nylon litterbags (8 sampling times × 3 litterbags for each time) were evenly placed on the ground within each plot on 6 January 2018 to perform field *in situ* litterbag decomposition experiments (Fig. S1E). Steel nails and stainless-steel wire were used to secure the litterbags to ensure that their 0.05 mm mesh size sides (litterbag mesh sizes, see below) were close to the ground. The total litterbags placed in the field site were 648 (24 litterbags for each plot × 27 plots) bags.

Specifically, to simulate the natural decomposition process in the field, a mixture of litter leaves of distinct species was used in this study. Given microorganisms and soil fauna are both critical to litter decomposition, we used litterbags of 0.05 mm mesh size on the bottom side and 3.0 mm mesh size on the reverse side to determine the biodegradation (Fig. S1D). The 0.05 mm mesh size can retain fragmented litter in the litterbags and the 3.0 mm mesh size allows microorganisms and most soil fauna to access the litterbag (Peng et al., 2019; Setala et al., 1996; Tie et al., 2021). The length and width of litterbags were both 20 cm.

Freshly fallen leaves were collected from the study forest from 8 October to 26 November 2017, encompassing the main season for litter fall (Fig. S1C) (Tie et al., 2022). The leaves collected from the field sites were transported to the laboratory, air-dried and separated by tree species. The mass ratios of the collected *S. superba*, *Q. serrata*, and *S. botryantha* leaves were 3:1:1. Then, fifteen grams of the air-dried litter (i.e., 9.0 g *S. superba* + 3.0 g *Q. serrata* + 3.0 g *S. botryantha*) was placed in each litterbag (Fig. S1D). A total of approximately 700 litterbags were filled in December 2017 (27 plots × 8 sampling times × 3 litterbags for each time + 10 bags for determining initial litter quality = 658 bags), and ten litterbags among them were randomly selected to determine the initial litter moisture concentration (drying at 65°C for 96 h; the moisture concentration was 8.1%) and the initial litter quality (Table S1).

Three levels of N addition (N0, no N fertilizer added; N10, added 10 g N m^{-2} y^{-1}; and N20, added 20 g N m^{-2} y^{-1}) and three levels of P addition (P0, no P fertilizer added; P5, added 5 g P m^{-2} y^{-1}; P15, added 15 g P m^{-2} y^{-1}) were used in the experiment. We used a factorial design, with nine treatments that included low and high N- and P-only addition treatments (i.e., N10P0, N20P0, N0P5, and N0P15), N and P coaddition treatments (i.e., N10P5, N20P5, N10P15, and N20P15), and a control treatment (i.e., N0P0). The N and P fertilizers were applied with ammonium nitrate (NH_4NO_3) and sodium dihydrogen phosphate (NaH_2PO_4), respectively. The levels of 10 and 20 g N m^{-2} y^{-1} additions were designed to simulate projected increases of atmospheric N deposition of approximately 100% and 200%, respectively, in the coming thirty years (2020-2050) in this rainy area (Tie et al., 2022; Tie et al., 2021; Tu et al., 2014). The levels of 5 and 15 g P m^{-2} y^{-1} were added to simulate different P availabilities. These levels of N and P addition were also applied by prior studies in (sub)-tropical forests of China (Chen et al., 2013; Mao et al., 2017; Zhang et al., 2020). NH_4NO_3 (i.e., 20.83 g N and/or 41.67 g N) and NaH_2PO_4 (i.e., 10.42 g P and/or 31.25 g P) were dissolved in 2 L of water and sprayed evenly onto the ground in each N and/or P addition plot using a hand-held sprayer approximately every 30 days from 7 January 2018 to 7 January 2020 (Fig. S1F). The control plots were sprayed with 2 L of water each time.

Sample collection

Litter samples were collected eight times (i.e., 7 April, 7 July and 7 October 2018; 6 January, 6 April, 7 July and 6 October 2019; and 7 January 2020). Three litterbags were randomly collected from each plot at each sampling time and then transported to the laboratory. The three litterbags collected from each plot were oven-dried (65°C for 96 h) prior to litter dry weight determination and were then passed through a 0.1-mm sieve prior to total C and nutrient concentration determinations. The litter total C concentration was determined by the dichromate oxidation-external heating method (Schinner et al., 1996). Litter subsamples were digested with 10 ml of a mixture of sulfuric acid (8 ml) and perchloric acid (2 ml) prior to determinations of the concentrations of litter total N, P, K, Na, Mg, manganese (Mn), zinc (Zn), and copper (Cu) using an automatic discontinuous chemical analyzer (Smart-Chem 200, Paris, France) (Cleveland, 2002).

Statistical analysis

The litter elemental stoichiometric ratios (i.e., C/N, C/P, and N/P ratios) were mole-based. The accumulated litter mass loss (M_m, % of the initial dry weight) and accumulated release rate of the litter elements (R_t, % of the initial content) was defined by the following equations 1 and 2, respectively (Berg, 2014):

$$M_m(\%) = \frac{W_0 - W_t}{W_0} \times 100 \qquad (1)$$

$$R_t(\%) = \frac{c_0 W_0 - c_t W_t}{c_0 W_0} \times 100 \qquad (2)$$

where c_0 and c_t are the litter element concentrations at the initial and tth sampling times (mg g^{-1}), and

W_0 and W_t are the litter dry weights at the initial and *t*th sampling times (g), respectively.

Statistical analyses were performed using SPSS 25.0 for Windows (SPSS Inc., Chicago, USA). The Shapiro–Wilk test and Levene's test were first applied to test the normality and homogeneity of variance of each variable. The Box–Cox method was then used to transform the variables with nonnormal distributions or unequal variances (i.e., N and P concentrations) to normal distribution. Three-way (N addition, P addition, and sampling time) repeated-measures ANOVA (RE-ANOVA) was used to determine the main effects of N addition, P addition, sampling time, and their interactions on the elemental concentrations and stoichiometric ratios of litter and the accumulated mass loss and release rate of litter elements during the study period. A post hoc comparison was applied using a general linear model (GLM) with Tukey's HSD test to examine the differences in the effects of the treatments on the response variables during the study period if the interaction between added N and added P was significant. In all these models, N addition and P addition were between-subjects factors, and sampling time was a within-subjects variable. The results were adjusted by applying the Greenhouse-Geisser method if Mauchly's test of sphericity was not satisfied.

Results

Dynamics of litter mass, C, N, and P

Across all treatments, the concentrations of C and N in the litter decreased overall as decomposition time increased (Fig. 1A and C), while the concentrations of P in the litter (Fig. 1E) and litter accumulated mass loss increased overall (Fig. S2A). At the twenty-fourth month, the concentrations of litter C, N, and P in the control were 332±8.38, 6.62±0.44, and 1.02±0.01 mg/g, respectively; and their accumulated release rates were 69.0±0.50%, 62.1±2.40%, and -10.3±0.92% of the initial values, respectively (Fig. 1B, D, and F).

The main effects of N addition, P addition, sampling time, and their interactive effects on the concentrations and accumulated release rates of litter C, N, and P during the study period were significant (Table S2; $p < 0.05$). Specifically, the concentrations of C and N in litter were higher (Fig. 2A and C; $p < 0.05$) and the accumulated release rates of litter C, N, and P were lower (Fig. 2B, D, and F; $p < 0.05$) in the N-only addition treatments (i.e., N10P0 and N20P0) than in the control treatment (i.e., N0P0). The concentrations of N and P in litter were higher ($p < 0.05$) and the accumulated release rates of litter C, N and P were lower ($p < 0.05$) in the P-only addition treatments (i.e., N0P5 and N0P15) than in the control treatment. However, the accumulated release rates of litter C and P were higher in the N and P coaddition treatments (i.e., N10P5, N20P5, N10P15, and N20P15) than at the same levels of N- and P-only addition treatments ($p < 0.05$). Moreover, the accumulated release rate of litter C was lower in the N20P0 treatment than in the N10P0 treatment ($p < 0.05$). The accumulated litter mass loss was lower in the N10P0, N20P0, N0P5, and N0P15 treatments than in the control treatment (Fig. S2B; $p < 0.05$), but was higher in the N10P5, N20P5, N10P15, and N20P15 treatments than at the same levels of N- and P-only addition treatments ($p < 0.05$).

Dynamics of litter elemental stoichiometric ratios

The litter C/N, C/P, and N/P ratios across all treatments decreased overall as decomposition time increased (Fig. 3A–C). The main effects of N addition, P addition, sampling time, and their interactive effects on the litter C/N ratio and N/P ratio during the study period were significant (Table S3; $p < 0.05$). Specifically, the C/P and N/P ratios in the litter were higher in the N-only addition treatments (i.e., N10P0 and N20P0) than in the control treatment (Fig. 4B and C; $p < 0.05$) and lower in the P addition plots (i.e., N0P5, N0P15, N10P5, N20P5, N10P15, and N20P15) than in the plots with no P added (i.e., N10P0 and N20P0) ($p < 0.05$).

Dynamics of litter metal nutrients

Across all treatments, the concentrations of K, Na, and Mn in the litter decreased as decomposition time increased (Fig. 5A, C, and G), while the concentration of Mg in the litter decreased over the first nine months of decomposition and then increased (Fig. 5E), and the concentrations of Zn and Cu in the litter first increased (over the first nine months of decomposition for Zn and first eighteen months of decomposition for Cu) and then decreased (Fig. 5I and K). At the twenty-fourth month, the order of the accumulated release rates of the litter elements in the control treatment was Mn (85.3±3.82%) > K (80.4±0.29%) > Na (63.1±1.09%) > Zn (43.8±9.12%) > Mg (39.4±1.61%) > Cu (-47±1.33%).

The main effects of N addition, P addition, sampling time, and their interactive effects on the concentrations and accumulated release rates of litter K, Na, Mg, and Mn during the study period were significant (Table S4; $p < 0.05$). Specifically, the accumulated release rates of litter K, Na, Mn, and Cu were lower (Fig. 6B, D, H, and L; $p < 0.05$) and the accumulated release rate of litter Mg was higher (Fig. 6F; $p < 0.05$) in the N-only addition treatments (i.e., N10P0 and N20P0) than in the control treatment. The accumulated release rates of litter Na and Mn were lower ($p < 0.05$) and the accumulated release rate of litter Mg was higher ($p < 0.05$) in the N0P15 treatment than in the control treatment. However, the accumulated release rates of Na, Mn, and Cu were higher (Fig. 6D and H; $p < 0.05$) and the accumulated release rate of litter Mg was lower ($p < 0.05$) in the N and P coaddition treatments (i.e., N10P5, N20P5, N10P15, and N20P15) than in the same level of N- and P-only addition treatments.

Discussion

Effects of N and P addition on litter C decomposition

In our study, the N-only addition treatments decreased the litter C release rate, supporting our first hypothesis, which was also consistent with prior studies (Bubier et al., 2007; Dias et al., 2013; Zhu et al., 2016a). The decreased litter C decomposition rate under N-only addition can be explained by a combination of multiple mechanisms. First, the products of lignin degradation can recombine with exogenous N to form lignin-like compounds via the participation of microorganisms (Berg and McClaugherty, 2008; Zhu et al., 2016b), suppressing the release of recalcitrant C from litter. Second, decreased biodegradation caused by soil acidification is a crucial reason why N addition usually inhibits litter C loss (Baiocchi Jacobson et al., 2011; Mao et al., 2017) because the addition of ammonium nitrate can decrease soil pH via stimulating the nitrification and reducing the soil exchangeable cation (e.g., Na^+ and K^+) contents (Xu et al., 2007). Third, the addition of only N most likely aggravated N and P imbalances and thus inhibited the biodegradation according to growth rate hypothesis (Allison et al., 2009; Sardans et al., 2011), resulting in a lower rate of litter C decomposition. However, Jiang et al. (2018) reported that N addition promoted litter C decomposition in a subtropical coniferous forest (*Pinus massoniana*), which was not in agreement with our results. The discrepancy between our study and the study by Jiang et al. (2018) may be closely linked to differences in litter traits (e.g., the litter in our study was broadleaved, while Jiang et al. (2018) studied a coniferous forest), as recent studies found that litter traits and N supply interactively drove litter decomposition (Song et al., 2019; Valera-Burgos et al., 2013). In addition, the accumulated release rate of litter C was lower in the high N-only addition (N20P0) treatment than in the low N-only addition (N10P0) treatment (Fig.

2B), suggesting that the negative effects of N-only addition treatments on litter C decomposition were enhanced with increasing levels of N addition.

Phosphorus is a key element in organism Pyranosyl-RNA (p-RNA) synthesis, which is an oligonucleotide important for phosphodiester groups (Elser et al., 2007). The prior studies, therefore, highlighted that the biogeochemical cycles of C can increase with elevated P availability (Baiocchi Jacobson et al., 2011; Elser et al., 2007; Sardans et al., 2011). Surprisingly, the treatments with P-only, in the present study, inhibited litter C decomposition (Fig. 2A and B), which was inconsistent with most prior P addition studies that highlighted the positive effects of P addition on litter C decomposition (Baiocchi Jacobson et al., 2011; Carate-Tandalla et al., 2018; Zhang et al., 2020). However, in a study conducted in a tropical forest, it was found that P addition decreased the rate of litter C loss (Chen et al., 2013), which was in line with our results. On the one hand, in our study the treatments with P-only increased the litter P concentration (Fig. 2E), which potentially had a positive effect on the biodegradation of litter C because more P could be allocated to rRNA for the synthesis of proteins in order to support the growth of organisms (Elser et al., 2007; Sardans et al., 2011). On the other hand, however, P-only addition treatments decreased the litter C/P ratio and N/P ratio during decomposition compared with the control treatment (Fig. 4B and C), which alleviated microbial P limitations based on the economic theory of microbial metabolism (Di Lonardo et al., 2018; Jiang et al., 2018). The alleviation of P limitation can reduce microbial P mining because sufficient P might be present for microorganisms to efficiently build new biomass and grow, which reduces the rate of litter organic matter decomposition (Jiang et al., 2018). Furthermore, our prior study highlighted that the treatments with P-only addition reduced the litter cellulase activity during decomposition due to decreasing soil pH (Tie et al., 2022), which also confirms there is a negative effect of P-only addition on the biodegradation of litter C. Overall, the negative effects associated with P-only additions in our study (e.g., decreased microbial P mining and exacerbated soil acidification) very likely overwhelmed the potential positive effects of increased P availability on litter C decomposition, thus decreasing the rate of litter C decomposition.

Nitrogen and P generally have synergistic effects on ecosystem processes, such as net primary productivity (Elser et al., 2007; Sardans et al., 2011), soil respiration (Wei et al., 2020), soil microbial degradation (Chen et al., 2013; Widdig et al., 2020), and soil fauna-induced litter decomposition in forest ecosystems (Pompermaier et al., 2021). Therefore, these ecosystem processes can usually be facilitated by the combined addition of N and P (Chen et al., 2013; Pompermaier et al., 2021; Wei et al., 2020; Widdig et al., 2020). In our study, the interaction of N addition and P addition on the concentration and accumulated release rate of litter C was significant, and their coaddition treatments increased litter C loss, which supported our second hypothesis predicting a positive synergistic effect of N addition and P addition and was consistent with prior studies (Chen et al., 2013; Zhang et al., 2020). This result can be explained by a stimulated biodegradation under the N and P coaddition treatments (Craine et al., 2007; Sardans et al., 2011). Our prior study showed that the N and P coaddition treatments increased litter MBC, litter C-hydrolase (e.g., invertase and cellulase) activities, and soil fauna diversity during decomposition (Tie et al., 2022), which strongly supported litter C biodegradation in the present study (Pompermaier et al., 2021; van Huysen et al., 2016). In short, the combined N and P addition treatments can increase the biological activities of soil fauna and microorganisms and the associated acceleration of litter C decomposition, which was consistent with our expectations and in agreement with the frame of ecological stoichiometry and growth rate hypothesis (Sardans et al., 2011; Sterner and Elser, 2002).

Effects of N and P addition on the release of litter nutrients

Nitrogen release from litter by leaching and biodegradation pathways is one of the drivers of forest N cycles (Berg, 2014; Peng et al., 2020). The N-only addition treatments in our study inhibited litter N loss during the study period (Fig. 2C and D), which was in agreement with most prior studies because exogenous N often accumulated in the litter, thus decreasing the litter N release rate (Baiocchi Jacobson et al., 2011; Zhang and Liu, 2019; Zhu et al., 2016b). Interestingly, the litter N release rate, however, was increased by the N and P coaddition treatments. This positive effect may be associated with increased N-hydrolase activity. For instance, recent studies highlighted that combined N and P addition increased the activities of urease and N-acetyl-*β*-D-glucosaminidase and thus accelerated net rates of N mineralization and nitrification (Chen et al., 2016a; Wang et al., 2020), which could further aggravate litter N biodegradation.

In our study, the amount of litter P in the control treatment was higher at the end of the study period than that in the initial litter (Fig. 1F), which was similar to the results reported by Ball et al. (2009). When litter-dwelling microorganisms are under nutrient-poor or nutrient-deficient conditions, fungal organisms can transfer limiting nutrients from the nutrient-rich interfaces (e.g., soil or fresh fallen leaves) to nutrient-poor interfaces (e.g., decomposed litter) via their mycelia (Bani et al., 2018; Duarte et al., 2010). Therefore, the content of nutrients that are originally low in the litter can increase or even become enriched as decomposition time increases (Bani et al., 2018; Chen et al., 2013; Duarte et al., 2010). In our prior study conducted in the same forest, we reported that litter acid phosphatase activity after the twelfth month of decomposition (>39.7±1.97 μmol g^{-1} h^{-1}) was three times higher than that at the beginning of decomposition (10.6±0.134 μmol g^{-1} h^{-1}) (Tie et al., 2022), indicating that the litter was P-deficit or P-limited during the late stage of decomposition (Elser et al., 2007; Sardans et al., 2011). Therefore, in the late stage, P can migrate and can be fixed into the decomposed litter via fungal activities (Zhang et al., 2020), resulting in increased P concentrations in the residual litter and decreased litter P accumulated release rate. Moreover, the N20P0 treatment, in this study, decreased the release rate of P from litter during the study period (Fig. 2F), which was consistent with results reported by Baiocchi Jacobson et al. (2011). This result was most likely related to exacerbated litter P limitations under N20P0 treatment. In our study, the N20P0 treatment increased the C/P and N/P ratios of the litter especially after the twelfth month of decomposition (Fig. 3B and C; Fig. 4B and C), which could result in higher P transfer and fixation by fungal mycelia due to increased litter P limitations during decomposition (Elser et al., 2007; Sardans et al., 2011; Yue et al., 2020), thus reducing the litter P release rate.

Three patterns for litter metal nutrient degradation exist, namely, direct release, enrichment-to-release, and release-to-enrichment (Baiocchi Jacobson et al., 2011; Moore et al., 2006; van Huysen et al., 2016). In this study, the concentrations of K and Na in the litter decreased rapidly during the early stage of decomposition (Fig. 5A and C), which was consistent with prior studies (Berg and McClaugherty, 2008; Zhang et al., 2020). However, the concentrations of Zn and Cu in the litter first increased and then decreased during decomposition (Fig. 5I and K). Two mechanisms could explain these different patterns. First, litter nutrients that are easily decomposed are often released directly, while recalcitrant elements accumulate during the early stage of decomposition (Baiocchi Jacobson et al., 2011; Moore et al., 2006; van Huysen et al., 2016). Zinc and Cu are more difficult to release than K and Na due to their lower solubility in water (Berg and McClaugherty, 2008; Sardans et al., 2011), which may have resulted in different release patterns among these elements. Second, K and Na mainly exist in the litter in the form of free ions, so they are quickly lost by leaching during the early stage of decomposition (Berg and McClaugherty, 2008). However, Zn and Cu are present in the form of protein chelates because they act as coenzymes; thus, proteins must undergo degradation before these elements are released (Berg and McClaugherty, 2008; Sardans et al., 2011). Consequently, litter Zn and Cu were mainly released via biodegradation, and their concentrations varied with biological activities, e.g., microbial mining (nutrient loss) and migration (nutrient fixation) (Yue et al., 2020; Zhang et al., 2020). Thus, in our study, the Zn concentration increase (Fig. 5I) and Cu accumulation in the residual litter (Fig. 5K and L) likely occurred because microbial migration was stronger than microbial mining effects (van Huysen et al., 2016; Yue et al., 2020). Additionally, Mg is easy to leach out from litter (Zhang et al., 2020), resulting in a decreased Mg concentration in litter over the first nine months of decomposition in the present study (Fig. 5E). Interestingly, the Mg concentration in residual litter increased at the late stage of the decomposition (Fig. 5E), which was in line with prior studies (Berg and McClaugherty, 2008; Yue et al., 2020). The increase in Mg concentration in residual litter may be a result of transfer and fixation by fungal mycelia (Dias et al., 2013; Yue et al., 2020). More studies, however, are needed to confirm it.

Globally, a substantial number of studies have reported the effects of N addition on the litter Mn release rate (Peng et al., 2022; Zhang and Liu, 2019; Zhou et al., 2021), but the underlying mechanisms remain unclear. Moreover, fewer studies have focused on the mechanism by which P addition influences the N addition effect on the release of litter Mn. In our study, the N- and P-only addition treatments decreased the litter Mn release rate, but the coaddition treatments increased the Mn release rate (Fig. 6G). These results were similar to those reported in prior studies in subtropical and temperate forests, which showed that N addition decreased the litter Mn loss rate (Yan et al., 2020; Zhou et al., 2021). One potential mechanism could explain this discrepancy. Fungi can synthesize Mn peroxidase to stimulate Mn release from litter (Bani et al., 2018; Berg and McClaugherty, 2008). Therefore, the litter Mn release rate usually correlates with microbial abundance and community composition (Wang et al., 2020). Our prior study showed that the litter MBC concentration during decomposition was decreased by N- and P-only addition treatments due to decreasing soil pH, while it was increased by N and P coaddition (Tie et al., 2021), which might have altered litter Mn microbial degradation. However, a field study in a subtropical forest reported that N addition (5 and 15 g N m^{-2} y^{-1}) increased the litter Mn release rate across the first two years of decomposition (Peng et al., 2022), which is not consistent with our result. A difference in litterbag mesh sizes used in the two field experiments may be a reason for the differing results between our study (0.05 mm mesh size on the bottom and 3.0 mm on the reverse) and the study by Peng et al. (2022) (mesh sizes on the bottom and reverse sides were both 1.0 × 1.0 mm). These findings highlight that mesh size and its mediation of soil faunal communities involved in litter decomposition is critical for the release of litter Mn, which was in line with a prior study in Hawaiian rainforest showing macro-fauna increased 30.3% rate of litter Mn release (Meyer et al., 2011). Therefore, we suggest incorporating soil fauna, especially those body widths larger than 1.0 mm, into the models for litter nutrient degradation responses to N and/or P addition in the future.

Conclusion

The N-only addition treatments reduced the litter mass loss and the release rates of litter C, N, P, K, Na, Mn, and Cu in the N-rich study forest, which was consistent with our expectations. The treatments with P-only decreased the litter mass loss and the release rates of litter C and nutrients (e.g., N, P, K, Na, and Mn) over the study period. Moreover, the N and P coaddition treatments accelerated the loss of litter mass and the release of litter C, N, K, Na, Mn, and Cu related to increases biological activities of soil fauna and microorganisms. Overall, the results of individual and combined additions of N or/and P strongly suggest that biological activity can be increased only when N and P are combined supplied, thus accelerating litter C decomposition and the release of nutrients (e.g., N, K, Na, Mn, and Cu), as expected within the frame of ecological stoichiometry and growth rate hypothesis. Finally, our study highlights that the effect of N addition on C and nutrients release from litter depends on P availability.

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Competing interests

The authors declare that they have no competing financial interests.

Contributions of the coauthors

Liehua Tie: Methodology, Writing - original draft, Writing - review & editing. **Shengzhao Wei:** Methodology, Writing - original draft, Writing - review & editing. **Josep Peñuelas:** Writing - original draft, Writing - review & editing. **Jordi Sardans:** Writing - original draft, Writing - review & editing. **Xing Liu:** Methodology, Writing - review & editing. **Shixing Zhou:** Methodology, Writing - review & editing. **Xiong Liu:** Methodology, Writing - review & editing. **Arun K. Bose:** Writing - original draft, Writing - review & editing. **Congde Huang:** Methodology, Writing - original draft, Writing - review & editing.

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Figure captions

Fig. 1 Dynamics of the litter carbon (C), nitrogen (N), and phosphorus (P) concentrations and their accumulated release rates under N and P addition. Values are the means ± standard deviations of three replicate plots.

Fig. 2 Effects of nitrogen (N) and phosphorus (P) addition on the mean litter carbon (C), N, and P concentrations and their accumulated release rates during two years of fertilization based on repeated-measures ANOVA. Data are from eight sampling times and nine treatments, and values are the means ± standard deviations of three replicate plots. Different capital letters denote significant differences between nitrogen addition treatments at the same level of phosphorus addition ($p < 0.05$), and different lowercase letters denote significant differences between phosphorus addition treatments at the same level of nitrogen addition ($p < 0.05$).

Fig. 3 Dynamics of the litter elemental stoichiometric ratios under nitrogen (N) and phosphorus (P) addition. Values are the means ± standard deviations of three replicate plots.

Fig. 4 Effects of nitrogen (N) and phosphorus (P) addition on the mean litter elemental stoichiometric ratios during two years of fertilization based on repeated-measures ANOVA. Data are from eight sampling times and nine treatments, and values are the means ± standard deviations of three replicate plots. Different capital letters denote significant differences between nitrogen addition treatments at the same level of phosphorus addition ($p < 0.05$), and different lowercase letters denote significant differences between phosphorus addition treatments at the same level of nitrogen addition ($p < 0.05$).

Fig. 5 Dynamics of the litter metal nutrient concentrations and their accumulated release rates under nitrogen (N) and phosphorus (P) addition. Values are the means ± standard deviations of three replicate plots.

Fig. 6 Effects of nitrogen (N) and phosphorus (P) addition on the mean litter metal nutrient concentrations and their accumulated release rates during two years of fertilization based on repeated-measures ANOVA. Data are from eight sampling times and nine treatments, and values are the means ± standard deviations of three replicate plots. Different capital letters denote significant differences between nitrogen addition treatments at the same level of phosphorus addition ($p < 0.05$), and different lowercase letters denote significant differences between phosphorus addition treatments at the same level of nitrogen addition ($p < 0.05$).

Fig. 1

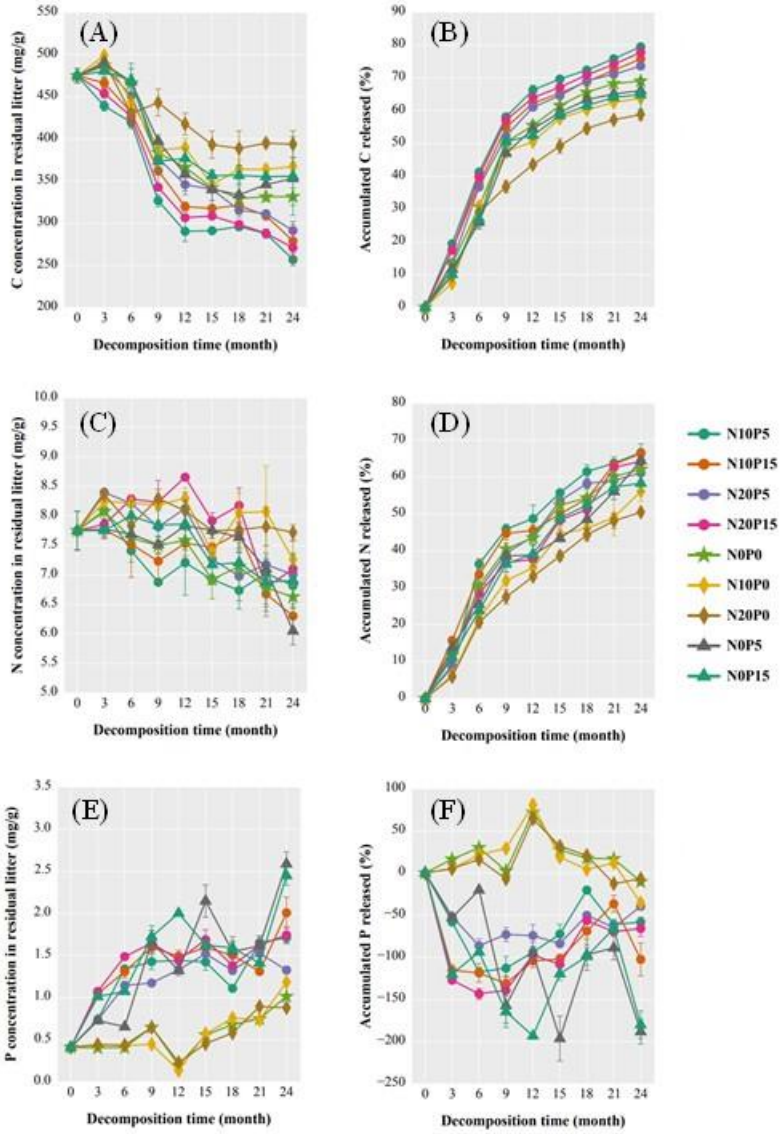

(A)
C concentration in residual litter (mg/g)
550
500
450
400
350
300
250
200
Decomposition time (month)
0
3
6
9
12
15
18
21
24
(B)
Accumulated C released (%)
90
80
70
60
50
40
30
20
10
0
Decomposition time (month)
(C)
N concentration in residual litter (mg/g)
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
Decomposition time (month)
(D)
Accumulated N released (%)
80
70
60
50
40
30
20
10
0
Decomposition time (month)
N10P5
N10P15
N20P5
N20P15
N0P0
N10P0
N20P0
N0P5
N0P15
(E)
P concentration in residual litter (mg/g)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Decomposition time (month)
(F)
Accumulated P released (%)
100
50
0
−50
−100
−150
−200
−250
Decomposition time (month)

Fig. 2

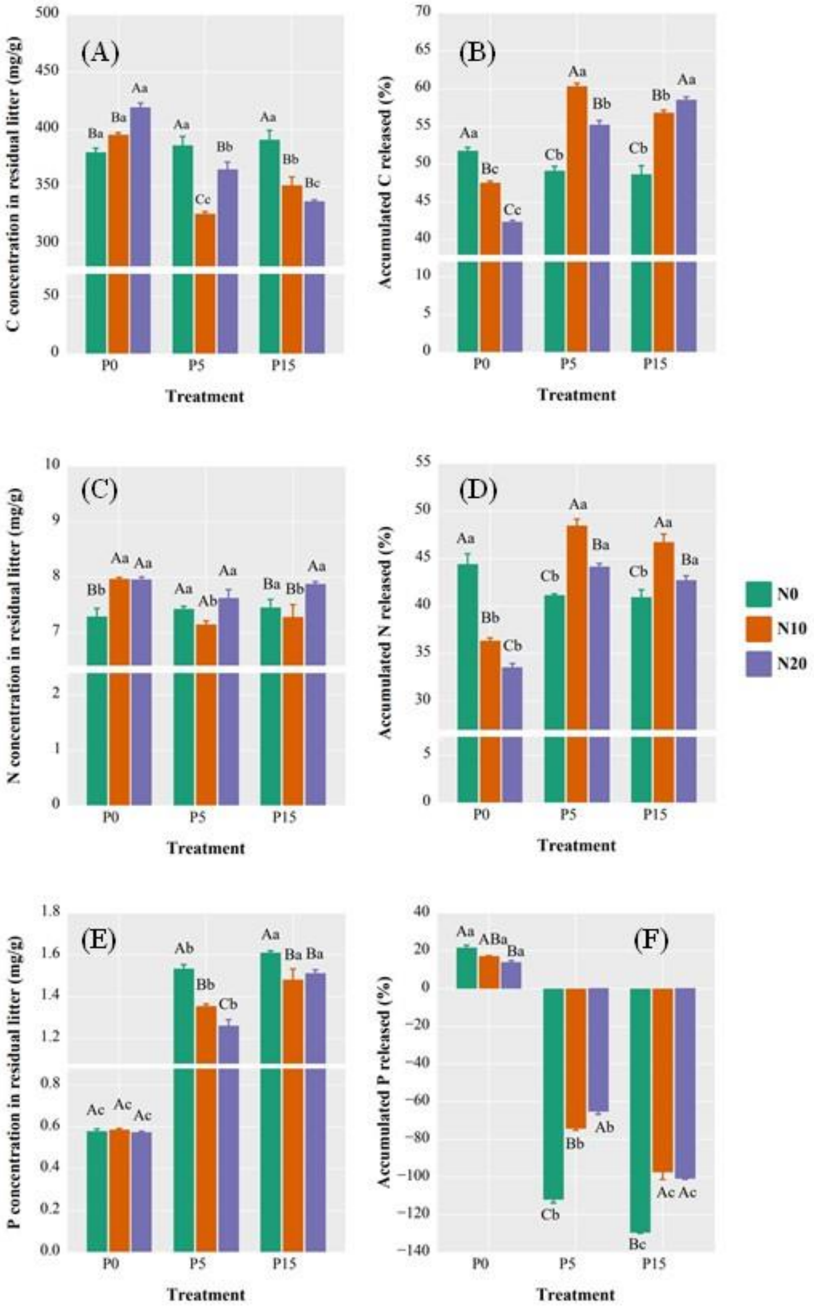

(A)
C concentration in residual litter (mg/g)
500
450
400
350
300
50
0
Ba Ba Aa
Aa Cc Bb
Aa Bb Bc
P0
P5
P15
Treatment
(B)
Accumulated C released (%)
70
65
60
55
50
45
40
10
5
0
Aa Bc Cc
Cb Aa Bb
Cb Bb Aa
P0
P5
P15
Treatment
(C)
N concentration in residual litter (mg/g)
10
9
8
7
2
1
0
Bb Aa Aa
Aa Ab Aa
Ba Bb Aa
P0
P5
P15
Treatment
(D)
Accumulated N released (%)
55
50
45
40
35
30
5
0
Aa Bb Cb
Cb Aa Ba
Cb Aa Ba
P0
P5
P15
Treatment
N0
N10
N20
(E)
P concentration in residual litter (mg/g)
1.8
1.6
1.4
1.2
0.8
0.6
0.4
0.2
0.0
Ac Ac Ac
Ab Bb Cb
Aa Ba Ba
P0
P5
P15
Treatment
(F)
Accumulated P released (%)
40
20
0
−20
−40
−60
−80
−100
−120
−140
Aa ABa Ba
Cb Bb Ab
Bc Ac Ac
P0
P5
P15
Treatment

Fig. 3

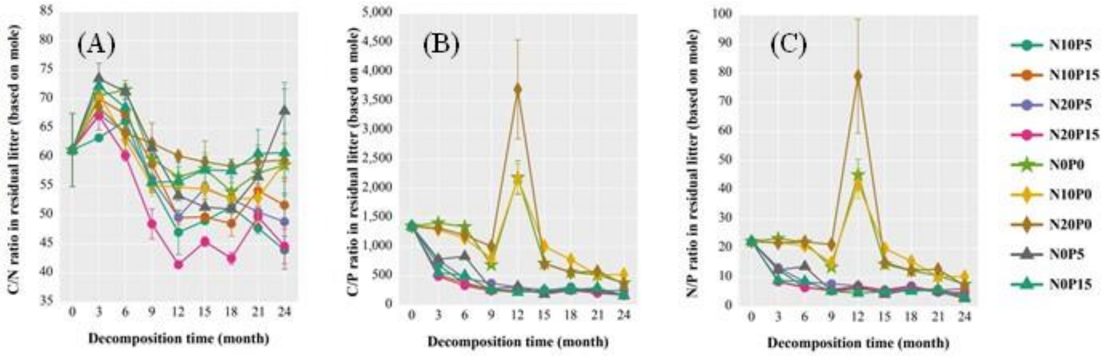

(A)
(B)
(C)
C/N ratio in residual litter (based on mole)
C/P ratio in residual litter (based on mole)
N/P ratio in residual litter (based on mole)
Decomposition time (month)
0
3
6
9
12
15
18
21
24
35
40
45
50
55
60
65
70
75
80
85
0
500
1,000
1,500
2,000
2,500
3,000
3,500
4,000
4,500
5,000
0
10
20
30
40
50
60
70
80
90
100
N10P5
N10P15
N20P5
N20P15
N0P0
N10P0
N20P0
N0P5
N0P15

Fig. 4

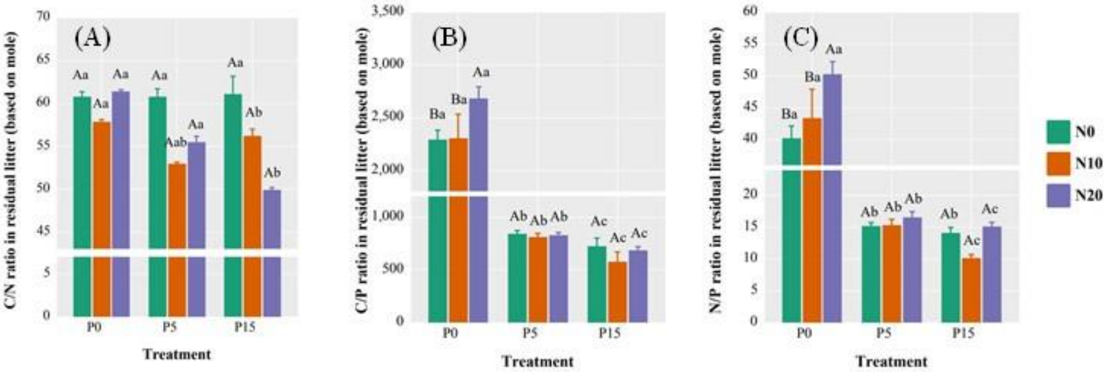

(A)
C/N ratio in residual litter (based on mole)
70
65
60
55
50
45
5
0
Aa
Aa
Aa
Aa
Aab
Aa
Aa
Ab
Ab
P0
P5
P15
Treatment
(B)
C/P ratio in residual litter (based on mole)
3,500
3,000
2,500
2,000
1,000
500
0
Ba
Ba
Aa
Ab
Ab
Ab
Ac
Ac
Ac
P0
P5
P15
Treatment
(C)
N/P ratio in residual litter (based on mole)
60
55
50
45
40
20
15
10
5
0
Ba
Ba
Aa
Ab
Ab
Ab
Ab
Ac
Ac
P0
P5
P15
Treatment
N0
N10
N20

Fig. 5

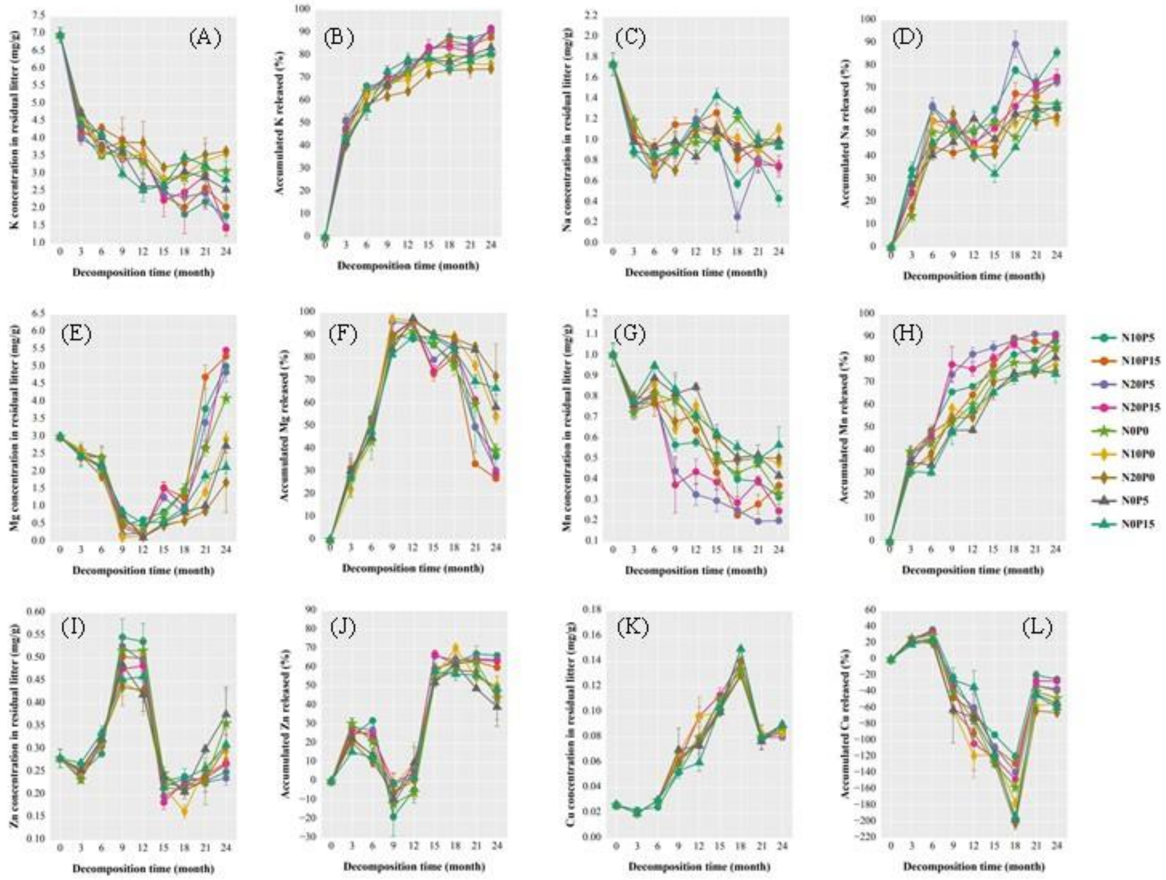
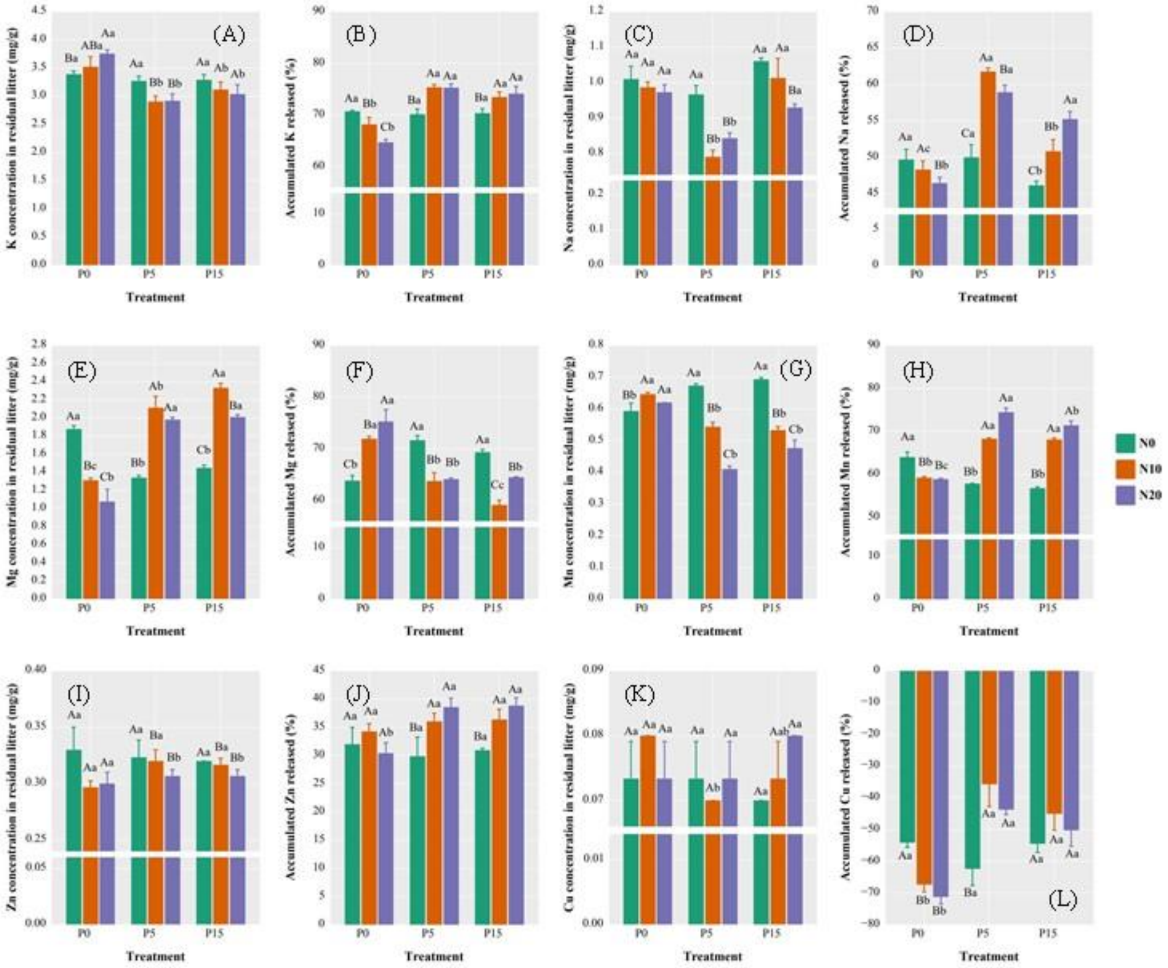

(A)
K concentration in residual litter (mg/g)
(B)
Accumulated K released (%)
(C)
Na concentration in residual litter (mg/g)
(D)
Accumulated Na released (%)
(E)
Mg concentration in residual litter (mg/g)
(F)
Accumulated Mg released (%)
(G)
Mn concentration in residual litter (mg/g)
(H)
Accumulated Mn released (%)
(I)
Zn concentration in residual litter (mg/g)
(J)
Accumulated Zn released (%)
(K)
Cu concentration in residual litter (mg/g)
(L)
Accumulated Cu released (%)
Decomposition time (month)
0 3 6 9 12 15 18 21 24
N10P5
N10P15
N20P5
N20P15
N0P0
N10P0
N20P0
N0P5
N0P15

Fig. 6


(A)
K concentration in residual litter (mg/g)
Ba ABa Aa Aa Bb Bb Aa Ab Ab
(B)
Accumulated K released (%)
Aa Bb Cb Ba Aa Aa Ba Aa Aa
(C)
Na concentration in residual litter (mg/g)
Aa Aa Aa Aa Bb Bb Aa Aa Ba
(D)
Accumulated Na released (%)
Aa Ac Bb Ca Aa Ba Cb Bb Aa
(E)
Mg concentration in residual litter (mg/g)
Aa Bc Cb Bb Ab Aa Cb Aa Ba
(F)
Accumulated Mg released (%)
Cb Ba Aa Aa Bb Bb Aa Cc Bb
(G)
Mn concentration in residual litter (mg/g)
Bb Aa Aa Aa Bb Cb Aa Bb Cb
(H)
Accumulated Mn released (%)
Aa Bb Bc Bb Aa Aa Bb Aa Ab
(I)
Zn concentration in residual litter (mg/g)
Aa Aa Aa Aa Ba Bb Aa Ba Bb
(J)
Accumulated Zn released (%)
Aa Aa Ab Ba Aa Aa Ba Aa Aa
(K)
Cu concentration in residual litter (mg/g)
Aa Aa Aa Aa Ab Aa Aa Aab Aa
(L)
Accumulated Cu released (%)
Aa Bb Bb Ba Aa Aa Aa Aa Aa
Treatment
P0
P5
P15
N0
N10
N20

Supplementary figures

Table S1 The initial litter carbon and nutrient concentrations and ratios.

Total C concentration (mg/g)	Total N concentration (mg/g)	Total P concentration (mg/g)	Lignin concentration (mg/g)	Cellulose concentration (mg/g)	C/N ratio (based on mole)	C/P ratio (based on mole)	N/P ratio (based on mole)
474 ± 8.60	7.75 ± 0.326	0.409 ± 0.010	301 ± 3.74	174 ± 3.97	61.2 ± 6.31	1159 ± 92.0	18.9 ± 1.5

Table S2 *F*- and *p value*s for repeated-measures ANOVA of the main effects of nitrogen (N) addition, phosphorus (P) addition, sampling time, and their interactions on the concentration and accumulated release rate of litter C, N, and P during two years of fertilization.

Variable	d.f.	*F values*	*p values*	Variable	d.f	*F values*	*p values*
C concentration (mg/g)				*Accumulated C released (%)*			
N effect (NE)	2	60.9	＜0.001	N effect (NE)	2	268	＜0.001
P effect (PE)	2	154	＜0.001	P effect (PE)	2	799	＜0.001
Time	7	605	＜0.001	Time	7	8489	＜0.001
NE × PE	4	76.2	＜0.001	NE × PE	4	436	＜0.001
NE × Time	14	3.67	＜0.05	NE × Time	14	12.5	＜0.001
PE × Time	14	7.99	＜0.001	PE × Time	14	10.7	＜0.001
NE × PE × Time	28	5.03	＜0.001	NE × PE × Time	28	6.21	＜0.001
N concentration (mg/g)				*Accumulated N released (%)*			
N effect (NE)	2	36.8	＜0.001	N effect (NE)	2	109	＜0.001
P effect (PE)	2	18.7	＜0.001	P effect (PE)	2	380	＜0.001
Time	7	93.2	＜0.001	Time	7	3370	＜0.001
NE × PE	4	17.0	＜0.001	NE × PE	4	232	＜0.001
NE × Time	14	2.21	＜0.05	NE × Time	14	4.82	＜0.001
PE × Time	14	6.69	＜0.001	PE × Time	14	7.11	＜0.001
NE × PE × Time	28	2.39	＜0.001	NE × PE × Time	28	10.0	＜0.001
P concentration (mg/g)				*Accumulated P released (%)*			
N effect (NE)	2	69.1	＜0.001	N effect (NE)	2	226	＜0.001
P effect (PE)	2	3934	＜0.001	P effect (PE)	2	6223	＜0.001
Time	7	388	＜0.001	Time	7	135	＜0.001
NE × PE	4	26.9	＜0.001	NE × PE	4	103	＜0.001
NE × Time	14	39.8	＜0.001	NE × Time	14	80.4	＜0.001
PE × Time	14	52.4	＜0.001	PE × Time	14	114	＜0.001
NE × PE × Time	28	19.8	＜0.001	NE × PE × Time	28	38.5	＜0.001

Data were obtained from eight sampling times and nine treatments.

Table S3 *F*- and *p value*s for repeated-measures ANOVA of the main effects of nitrogen (N) addition, phosphorus (P) addition, sampling time, and their interactions on the litter elemental stoichiometric ratios during two years of fertilization.

Variable	d.f.	*F value*s	*p value*s	Variable	d.f.	*F value*s	*p value*s
C/N ratio (based on mole)				*C/P ratio (based on mole)*			
N effect (NE)	2	108	＜0.001	N effect (NE)	2	1.66	0.219
P effect (PE)	2	61.9	＜0.001	P effect (PE)	2	823	＜0.001
Time	7	443	＜0.001	Time	7	4167	＜0.001
NE × PE	4	46.2	＜0.001	NE × PE	4	7.84	＜0.05
NE × Time	14	2.28	＜0.05	NE × Time	14	69.3	＜0.001
PE × Time	14	6.23	＜0.001	PE × Time	14	269	＜0.001
NE × PE × Time	28	4.08	＜0.001	NE × PE × Time	28	17.2	＜0.001
N/P ratio (based on mole)							
N effect (NE)	2	6.28	＜0.05				
P effect (PE)	2	572	＜0.001				
Time	7	3730	＜0.001				
NE × PE	4	8.98	＜0.001				
NE × Time	14	43.4	＜0.001				
PE × Time	14	245	＜0.001				
NE × PE × Time	28	16.9	＜0.001				

Data were obtained from eight sampling times and nine treatments**.**

Table S4 *F*- and *p value*s for repeated-measures ANOVA of the main effects of nitrogen (N) addition, phosphorus (P) addition, sampling time, and their interactions on the concentration and accumulated release rate of litter metal nutrients during two years of fertilization.

Variable	d.f.	*F value*s	*p value*s	Variable	d.f.	*F value*s	*p value*s
K concentration (mg/g)				*Accumulated K released (%)*			
N effect (NE)	2	4.40	＜0.05	N effect (NE)	2	14.0	＜0.05
P effect (PE)	2	66.0	＜0.001	P effect (PE)	2	142	＜0.001
Time	7	147	＜0.001	Time	7	1010	＜0.001
NE × PE	4	11.8	＜0.001	NE × PE	4	48.3	＜0.001
NE × Time	14	7.91	＜0.05	NE × Time	14	8.92	＜0.001
PE × Time	14	9.53	＜0.001	PE × Time	14	7.84	＜0.001
NE × PE × Time	28	3.12	＜0.001	NE × PE × Time	28	3.70	＜0.001
Na concentration (mg/g)				*Accumulated Na released (%)*			
N effect (NE)	2	52.7	＜0.001	N effect (NE)	2	81.4	＜0.001
P effect (PE)	2	114	＜0.001	P effect (PE)	2	198	＜0.001
Time	7	110	＜0.001	Time	7	742	＜0.001
NE × PE	4	16.1	＜0.001	NE × PE	4	59.0	＜0.001
NE × Time	14	26.8	＜0.001	NE × Time	14	25.3	＜0.001
PE × Time	14	34.5	＜0.001	PE × Time	14	28.3	＜0.001
NE × PE × Time	28	12.9	＜0.001	NE × PE × Time	28	14.4	＜0.001
Mg concentration (mg/g)				*Accumulated Mg released (%)*			
N effect (NE)	2	84.1	＜0.001	N effect (NE)	2	27.7	＜0.001
P effect (PE)	2	177	＜0.001	P effect (PE)	2	76.8	＜0.001
Time	7	1405	＜0.001	Time	7	1806	＜0.001
NE × PE	4	185	＜0.001	NE × PE	4	96.3	＜0.001
NE × Time	14	43.0	＜0.001	NE × Time	14	24.4	＜0.001
PE × Time	14	33.9	＜0.001	PE × Time	14	17.5	＜0.001
NE × PE × Time	28	44.2	＜0.001	NE × PE × Time	28	27.3	＜0.001
Mn concentration (mg/g)				*Accumulated Mn released (%)*			
N effect (NE)	2	286	＜0.001	N effect (NE)	2	308	＜0.001
P effect (PE)	2	82.3	＜0.001	P effect (PE)	2	165	＜0.001
Time	7	557	＜0.001	Time	7	1984	＜0.001
NE × PE	4	114	＜0.001	NE × PE	4	208	＜0.001
NE × Time	14	19.9	＜0.05	NE × Time	14	22.0	＜0.001
PE × Time	14	7.61	＜0.001	PE × Time	14	6.04	＜0.001
NE × PE × Time	28	13.3	＜0.001	NE × PE × Time	28	11.2	＜0.001

Continued

Zn concentration (mg/g)				*Accumulated Zn released (%)*			
N effect (NE)	2	12.0	＜0.05	N effect (NE)	2	25.9	＜0.001
P effect (PE)	2	2.30	0.124	P effect (PE)	2	9.60	＜0.001
Time	7	949	＜0.001	Time	7	1420	＜0.05
NE × PE	4	2.51	0.064	NE × PE	4	9.44	＜0.001
NE × Time	14	7.92	＜0.001	NE × Time	14	5.92	＜0.001
PE × Time	14	6.72	＜0.001	PE × Time	14	7.14	＜0.001
NE × PE × Time	28	8.50	＜0.001	NE × PE × Time	28	6.71	＜0.001
Cu concentration (mg/g)				*Accumulated Cu released (%)*			
N effect (NE)	2	1.01	0.394	N effect (NE)	2	12.4	＜0.05
P effect (PE)	2	3.30	0.053	P effect (PE)	2	65.4	＜0.001
Time	7	2330	＜0.001	Time	7	1340	＜0.001
NE × PE	4	5.34	＜0.05	NE × PE	4	31.6	＜0.001
NE × Time	14	7.62	＜0.001	NE × Time	14	9.52	＜0.001
PE × Time	14	2.22	＜0.05	PE × Time	14	3.61	＜0.001
NE × PE × Time	28	6.40	＜0.001	NE × PE × Time	28	7.22	＜0.001

Data were obtained from eight sampling times and nine treatments.

Figure

Fig. S1 Schematic diagram of the study site and experimental photos. Panel A, satellite image of the study site; Panel B, the stand characteristics of the study forest; Panel C, collected fresh fallen leaves; Panel D, close-up image of the litterbag (length and width 20 × 20 cm; 0.05 mm mesh size on the bottom and 3.0 mm on the reverse) used in this study; Panel E, the litterbags placed on the ground in the field plot; Panel F, nitrogen and/or phosphorus fertilizations applied using a hand-held sprayer (3 L maximum capacity). The area within the red line in panel A is the rainy zone of southwestern China.

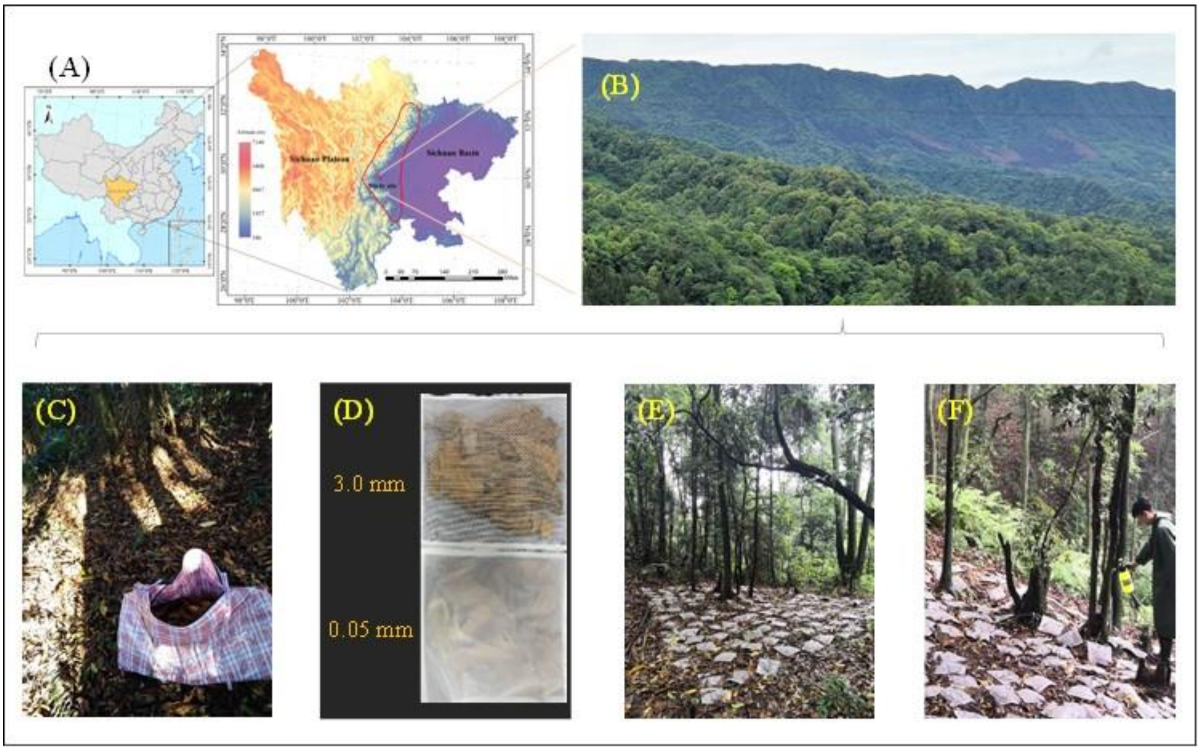


Fig. S2 Dynamic of the litter mass loss (A) and effects of nitrogen (N) and phosphorus (P) addition on the mean litter mass loss during two years of fertilization based on repeated-measures ANOVA (B). Data are from eight sampling times and nine treatments, and values are the means ± standard deviations of three replicate plots. Different capital letters denote significant differences between nitrogen addition treatments at the same level of phosphorus addition ($p < 0.05$), and different lowercase letters denote significant differences between phosphorus addition treatments at the same level of nitrogen addition ($p < 0.05$).